SUPPLEMENTARY MATERIAL

MATERIALS AND METHODS

DNA samples

Genomic DNA was extracted from peripheral blood lymphocytes following methodology described in Miller *et al*[1] or using the Autopure LS (Qiagen/Gentra, Germany) according to the manufacturer's protocol. DNA control samples ethnically matched to family ACS1 (n=275) were obtained from the Human Genome Research Center at USP.

Sanger sequencing

The eight coding exons and 5'UTR of the *GNAI3* gene (RefSeq NM_006496.3) were sequenced for seven non-affected and three affected patients (the only family members whose DNA was available for study) in family ACS1 and for cases Sp1 and Sp2. Primers sequences are listed in Supplementary Table S2. Sanger sequencing was performed according to standard techniques. dbSNP137 via the UCSC browser (http://genome.ucsc.edu/) and the Exome Variant Server (http://evs.gs.washington.edu/EVS/) were used to screen known polymorphisms.

Microsatellite analysis

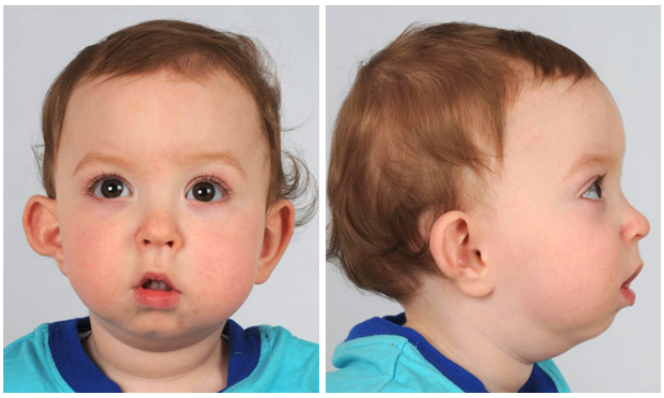
Fragment analysis of microsatellites for paternity testing was performed by standard techniques, using FAM or NED fluorescent dye-labelled oligos (Eurogentec, Belgium) and run on a 3130xl Genetic Analyzer (Applied Biosystems, California). The

following autosomal microsatellites were analyzed: D1S2696, D4S3038, D6S1572, D8S1836, D9S286, D11S4175, D14S1023, D16S3024, D19S412, D20S194, D21S1903.

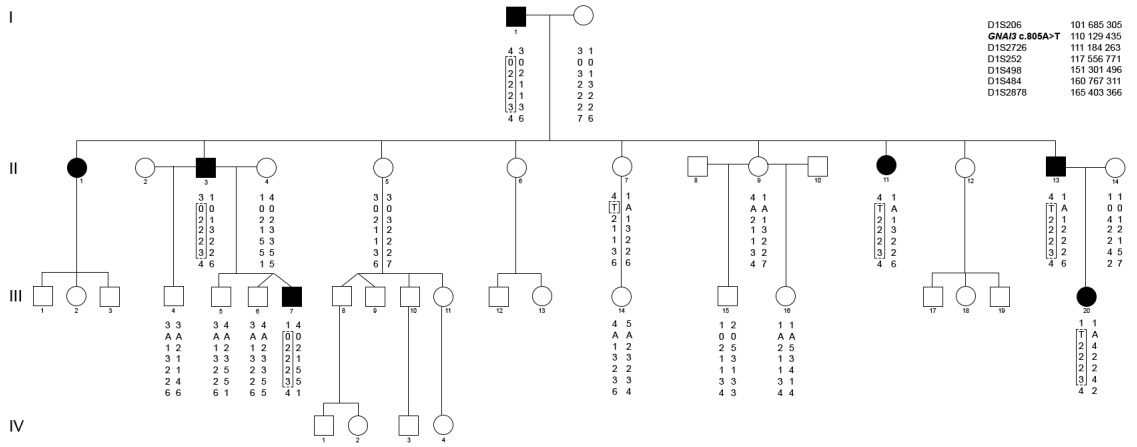
Variant analysis

Conserved protein domains were visualized via the Conserved Domain Database (CDD).[2] Evolutionary sequence conservation was assessed using the Vertebrate Multiz Alignment & Conservation track at the UCSC browser. The functional effect of variants was predicted using the programs PolyPhen-2 (http://genetics.bwh.harvard.edu/pph2/)[3] and SIFT (http://sift.jcvi.org/www/SIFT_enst_submit.html).[4] Protein modeling was conducted using Swiss-PdbViewer (Protein Data Bank structure ID: GNAI3, 2ODE) (http://www.expasy.org/spdbv/).[5]

SUPPLEMENTARY FIGURES AND TABLES



Supplementary Figure S1. Craniofacial features of patient Sp2.



Supplementary Figure S2. Pedigree of the ACS1 family showing reconstruction of previously published haplotypes of the chromosome 1 linkage region, along with the *GNAI3* genotypes. Microsatellite markers and *GNAI3* variant, with their respective physical positions (bp) (GRCh37/hg19), are provided at the top right of the figure. At-risk haplotypes are indicated inside a box; a dashed line indicates the possible location of the recombination point. Note that individual II-7 shares only a proximal region of the at-risk haplotype. Number 0 (zero): genotype for the *GNAI3* variant was not obtained because the DNA sample was not available; A (adenine) refers to the wild type allele; T (thymine) is the allelic variant.

Supplementary Table S1: Summary of the clinical features of ACS1 family, Sp1 and Sp2 ACS cases.

	Family ACS1	Sp1	Sp2
GENERAL			
Publication	Guion-Almeida *et al* , 2002	Propst *et al* , 2013	Present case
Sex	5 M, 3 F	M	M
Recurrence	+	-	-
DNA heterozygous variant	*GNAI3* : c.805A>T	*GNAI3* : c.134G>T	*GNAI3* : c.143C>A
FACE			
Round face	2/8	+	+
Full cheeks	3/8	+	+
Temporal bone anomalies	NR	+	NR
MOUTH			
Microstomia	2/8	+	+
Abnormal palate or uvula	1/7	-	+
Tongue polyp	NR	-	+
Glossoptosis	1/8	+	+
MANDIBLE			
Micrognathia	6/8	+	+
Mandibular condyle abnormality	1/8	+	+
EARS			
Auricular malformations	7/8	+	+
Hearing loss (conductive or sensorineural)	2/8	+	+
Preauricular pit	1/8	-	+
EYES			
Upslanting palpebral fissure	1/8	-	-
OTHER			
Respiratory distress	2/8	+	+
Feeding difficulties	1/8	+	+
Obstrutive sleep apnea	NR	+	+
Systolic murmur	NR	-	+
Umbilical hernia	1/8	-	-

M, male; F, female; +, present sign; -, absent sign; NR, not reported.

Supplementary Table S2: Primers used for amplification of *GNAI3* exons prior to Sanger sequencing: At the 5' extremity of forward primers the M13 tail 5' TGTAAAACGACGGCCAGT 3' was added; at the 5' extremity of reverse primers the M13 tail 5' CAGGAAACAGCTATGACC 3' was added. These M13 tails were designed to function as generic primers in the sequencing reaction. All primers work in a touchdown reaction, where, for primer pairs of exons 1 to 4, 6 and 8, the first fourteen cycles of the program have an annealing temperature of 62ºC and the twenty subsequent cycles an annealing temperature of 55ºC. For primer pairs of exons 5 and 7 the annealing temperature is 65ºC and 58ºC in the first 14 cycles and in the subsequent 20 cycles, respectively. All reactions were performed with touchdown -0.5ºC/cycle.

GNAI3	**Primer Foward 5'-3'**	**Primer Reverse 5'-3'**
Exon 1 (*)	TGGTTTAAACGGTGAAGGCTGCG	CACGCCCTCCAGATCCCTTTAG
Exon 2-3	GATACCAAGCAAGCCCTAGGACACT	ATCCCAGAAACCTACCGTGCACC
Exon 4	TCTGGGGATGGTGGTGTGTGG	GGTGCCAAGTCTCCCATTTACATTC
Exon 5	GCTAGGACTACAGGCATGCACCAC	GATGTTTGGGCCCCCATTCAG
Exon 6	TTCCCCTCCTTCCTTTAATCCATTTC	AGGTAGGAGGAAGGGCTGGCTAAC
Exon 7	TTCCTCCTACCTCCTCTCCCCCAA	GATATGGCACTCTTGACTTAGGGGC
Exon 8 (**)	AGAGTCCACTCTCACGAAGAACCTAA	GCAGCACCTCAACTTCACATCTTCC

(*) this primer pair includes part of the 5'UTR and the entire exon 1;
(**) this primer pair includes the entire exon 8 and part of the 3'UTR.

References

1. Miller, S. A., Dykes, D. D., Polesky, H. F. A simple salting out procedure for extracting DNA from human nucleated cells. *Nucleic Acids Res.* 1988; **16**: 1215.
2. Marchler-Bauer, A., Zheng, C., Chitsaz, F., *et al.* CDD: conserved domains and protein three-dimensional structure. *Nucleic Acids Res.* 2013; **41**: D348-52.
3. Adzhubei, I. A., Schmidt, S., Peshkin, L., *et al.* A method and server for predicting damaging missense mutations. *Nat. Methods* 2010; **7**: 248-9.
4. Ng, P. C., Henikoff, S. SIFT: predicting amino acid changes that affect protein function. Nucleic Acids Res. 2003; **31**: 3812-3814.
5. Guex, N., Peitsch, M. C. SWISS-MODEL and the Swiss-PdbViewer: An environment for comparative protein modeling. *Electrophoresis* 1997; **18**: 2714-2723.